Immunization of cattle against East Coast fever using *Theileria parva* (Marikebuni) and relaxation of tick control in North Rift, Kenya

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ABSTRACT

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A total of 90 animals was immunized against East Coast fever (ECF) using *Theileria parva* (Marikebuni) stock on three large-scale farms in Kiminini Division, Trans-Nzoia District, North Rift, Kenya. Another 90 cattle served as non-immunized controls. Following immunization the number of cattle with significant indirect fluorescent antibody (IFA) titres increased from 43.9 % to 84.4 % and 6.7 % of the cattle developed clinical ECF reactions. Two months after immunization, the immunized and non-immunized cattle were divided into two groups one of which was dipped every 3 weeks and the other dipped when total full body tick counts reached 100. All the animals were monitored for 51 weeks for incidences of ECF and other tick-borne diseases. Twenty-four cases of ECF were diagnosed among the non-immunized cattle compared to four cases among the immunized cattle; a difference that was significant ($P > 0.05$). There was no significant difference in the incidences of babesiosis and anaplasmosis between the immunized and non-immunized cattle.

Keywords: East Coast fever, immunization, *Theileria parva*, IFA, tick-borne diseases, tick control

INTRODUCTION

East Coast fever (ECF) which is caused by the protozoan parasite, *Theileria parva* and transmitted by the brown ear tick *Rhipicephalus appendiculatus*, is the major disease of cattle which limits dairy and beef production in parts of Eastern, Central and Southern Africa (Mukhebi 1992). Conventional control methods of the disease rely on regular and intensive use of chemical acaricides to control the tick vector. Acaricides are expensive to apply, may be a cause of environmental pollution, and with time ticks do develop resistance to acaricides (Nolan 1990). In addition, intensive application of acaricides can lead to a state of endemic instability to *Theileria parva* infections, so that in case of disruption of tick control, high mortalities from ECF could occur (Lawrence, Foggin & Norval 1980). Chemotherapeutic drugs, although expensive, can be used to treat infected animals when applied early in the disease (Dolan, Young, Leitch & Stagg 1984) .

An alternative ECF control method which involves an infection and treatment method of immunization has proved efficacious both at laboratory and field level (Radley, Brown, Burridge, Cunningham, Kirimi, Purnnel & Young 1975; Young, Leitch, Dolan, Mbogo, Ndungu, Grootenhuis & De Castro 1990). The method involves infection of an animal with live *Theileria parva* sporozoite stabilates and simultaneous treatment with an oxytetracycline preparation (Radley 1981). The resulting immune response protects the

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animals against homologous challenge for up to 3.5 years (Burridge, Morzaria, Cunningham & Brown 1972). In case of repeated tick infections in the field following immunization, this immunity may be life long. Currently, this is the only practical method available for immunization against ECF. A Kenyan stock of *Theileria parva* (Marikebuni) isolated from the Kenyan coast (Irvin, Dobbelaere, Mwamachi, Minami, Spooner & Ocama 1983) was found to confer protection in laboratory studies against several *Theileria parva* stocks isolated from different areas of Kenya (Mutugi, Young, Maritim, Ndungu, Mining, Linyonyi, Ngumi, Leitch, Morzaria & Dolan 1989). Field studies with this stock also showed that it was efficacious in Coastal and Central Kenya (Morzaria, Irvin, Taracha, & Spooner 1985; Mutugi, Ndungu, Linyonyi, Maritim, Mining, Ngumi, Kariuki, Leitch, D'Souza, Maloo & Lohr 1991; Mbogo, Kariuki, Ngumi, Rumberia, Linyonyi, Wanjohi, & Lesan 1994). Based on these findings, this parasite was recommended as the immunizing stock in Kenya.

Besides reduced mortality, the other major benefit to farmers following ECF immunization is the reduction in costs attributed to tick control and an increase in cattle productivity (Young, Mutugi, Kariuki, Lampard, Maritim, Ngumi, Linyonyi, Leitch, Ndungu, Lesan, Mining, Grootenhuis, Orinda & Wesonga 1992; Morzaria, Irvin, Wathanga, D'Souza, Katende, Young, Scott & Gettinby 1988). However, one of the problems that may arise with a relaxed tick-control policy is an increase of other tick-borne diseases such as anaplasmosis, babesiosis and heartwater. Although anedoctal evidence reveals that adoption of relaxed tick control is widely practised after ECF immunization, there is scanty and no reliable information on the optimum dipping intervals that should be adopted. Results from a simulated study showed that the greater the reduction in the use of acaricides after immunization, the higher the profitability achieved (Nyangito, Richardson, Mukhebi, Mundy, Zimmel, Namken & Perry 1994). However, total relaxation has not been advocated owing to an expected rise in the incidence of other tick-borne diseases (Mukhebi, Kariuki, Mussukuya, Mullins, Ngumi, Thorpe & Perry 1995; Morzaria *et al.* 1988).

The objective of this study was to evaluate the efficacy of *Theileria parva* (Marikebuni) stock in another Province of Kenya away from its isolation site as a prelude to the countrywide transfer of the technology to the field. The second objective was to determine the effect of reduced tick control on the incidence of ECF and other tick-borne diseases following ECF immunization. Information resulting from the latter objective would be used by the Department of Veterinary Services in Kenya to formulate a revised tick-control policy in areas where ECF immunization is practised.

MATERIALS AND METHODS

Trial site

The selection criteria of the trial site included availability of at least 200 cattle that could be used in the experiment in a major dairy farming area, farms on which there was a reliable tick-control programme (functional dip), a high-potential incidence of ECF based on seroprevalence, morbidity and mortality rate records, availability of farm infrastructures, farmers' consent for immunization and acceptance of associated risks such as loss of milk and other reactions during the immunization phase. A sample of approximately 30 randomly selected cattle on the farms which met the required criteria were bled and their antibodies to ECF determined using the indirect fluorescent antibody (IFA) test (Burridge & Kimber 1972). Only farms with 20–50 % seropositivity to *Theileria parva* were considered. Based on these criteria, one site was identified in Trans-Nzoia District, North Rift, Kenya.

Three farms were identified in the Kiminini Division of Trans-Nzoia District for the study. The trial site lies at 00°57'S, 00°34'E, with an altitude of 1 800–1 900 m above sea level. It receives a mean annual rainfall of 1 000–1 200 mm and the mean annual temperature is between 18 and 19 °C. The soils are clay-loam ferrasols and the vegetation cover consists of acacias, wire grass and introduced fodder grasses. The area falls under the Low Highlands subhumid (LH3) agro-ecological zone (Jaetzold & Schmidt 1983). The trials were conducted on Nyairo, Lulu and Milimo farms which were coded as farms 1, 2 and 3, respectively.

Farm 1

The farm is approximately 445 ha in extent. The major activity on the farm is the growing of crops, especially maize. A herd of 333 cattle, mainly of Friesian breed, were maintained in a free-grazing system at the start of the trial. The encroachment of grazing fields by animals from neighbouring farms is of common occurrence. One case of ECF and nine cases of anaplasmosis had been reported during the previous year. Ticks were controlled by dipping cattle weekly in Amitraz (Triatix®, Coopers, Kenya Ltd). A total of 100 animals were recruited for the trial.

Farm 2

This farm is approximately 514 ha in size with crop farming as the major activity. There were 159 animals, mainly of Friesian and Ayrshire breeds and their crosses which were kept in a free-grazing system at the beginning of the trial. The pastures are surrounded by maize fields. Twelve cases of ECF and one case of anaplasmosis were recorded during the previous year. Tick control was done by dipping every

10 days in Amitraz (Bovitraz®, Farmchem Kenya Ltd). Forty animals were recruited into the trial.

Farm 3

The farm is approximately 142 ha in extent. The major activity on the farm is crop farming, and a herd of 132 cattle, mainly of Friesian and Ayrshire breeds, were maintained in a free-grazing system at the commencement of the trial. The pastures are surrounded by maize fields. Ticks were controlled by weekly dipping with Amitraz (Triatix®, Cooper Kenya Ltd). In the previous year four cases of ECF, three of anaplasmosis and two of heartwater were reported. Forty animals were recruited into the trial.

Trial design

The trial animals comprised weaned calves and yearlings, and were randomized into four treatment groups each of which consisted of 45 animals. During the randomization, blocking was done using body weights. The cattle in the four groups received the following treatment:

Group 1 Immunized and dipped every 3 weeks (strategic dipping = SD)

Group 2 Immunized and dipped when the total number of ticks counted on half of the body reached/exceeded 50 on an individual animal in the group (tactical dipping = TD)

Group 3 Not immunized but dipped every 3 weeks (strategic dipping = SD)

Group 4 Not immunized but dipped when the total number of ticks counted on half of the body reached/exceeded 50 on an individual animal in the group (tactical dipping = TD)

Immunization

Theileria parva (Marikebuni), the stabilate used in this trial has been described in various studies (Irvin *et al.* 1983; Mutugi *et al.* 1989; Morzaria, Irvin, Taracha, Spooner, Voight, Fujinaga & Katende 1987). The stabilate was stored in 0.5 mℓ aliquotes in plastic straws kept under liquid nitrogen canisters. The plastic straws were removed from the container, rapidly thawed by rubbing them between the palms of the hand and dispensed into a universal bottle. Dilutions of 1 in 40 ($10^{-1.6}$) were made using Eagles Minimum Essential Medium with 3.5 % w/v bovine plasma albumin and 7.5 % v/v glyceral. After 30 minutes equilibration the stabilate was inoculated subcutaneously in the vicinity of the prescapular lymph node. A long acting oxytetracycline (Tetroxy LA, Bimeda) was administered at a dosage rate of 20 mg per kg body weight by deep intramuscular injection. The rectal temperatures of the animals were taken on alternate days between days 14 and 28 post immunization. Blood and lymph node smears were made from any immunized animals showing a febrile reaction (rectal temperature $\geq$39.5 °C). These smears were fixed in methanol, stained in Giemsa stain and examined for the presence of parasites. Any immunized animal developing clinical symptoms of ECF with fever and machroschizonts in lymph node smears for at least 3 days was designated as an "ECF reactor" and treated with buparvaquone (Butalex®, Pitman Moore, UK) at a dosage rate of 2.5 mg per kg body weight.

Animal monitoring following immunization

This phase started on day 60 post-immunization and lasted for 51 weeks. Clinical surveillance was kept on all the cattle in the experiment on a daily basis. Early ECF signs looked for included pyrexia, enlargement of superficial lymph nodes and dyspnoea. Needle biopsies from a prescapular lymph node and blood smears were made from all animals reported ill (especially when accompanied by a rectal temperature of $\geq$ 39.5 °C). Tissue smears on glass microscope slides were made from the lymph node smears, and these and the blood smears were fixed in methanol, stained in Giemsa stain and examined under a light microscope .The lymph node smears were examined for the presence of schizonts, while the blood smears were examined for piroplasms and other haemoparasites. Animals found to be suffering from ECF were treated with buparvaquone (Butalex®, Pitman Moore, UK) and supportive antibiotic drugs while cases of anaplasmosis or babesiosis were treated with imidocarb dipropionate (Imizol®, Pitman Moore, UK) according to the manufacturer's instructions. All animals were dewormed at the beginning of the trial and after 6 months with oxfendazole (Systemix®, Coopers, Kenya Ltd) at a dosage rate of 1 mℓ per 9 kg body weight.

Tick counts

Tick counts were done every 3 weeks immediately before dipping—total numbers and tick species were determined on half of the body of at least 20 randomly selected animals per treatment group. The full-body tick counts were estimated by multiplying the half-body counts by 2. Tick identification was done visually during the tick counts

Dip (acaricide) concentration

All the dips were emptied at the beginning of the tick-challenge period and replenished with Amitraz (Triatix®, Coopers, Kenya Ltd). Dip samples were tested every 3 weeks and dipping fluid concentrations were maintained at the correct level according to manufacturers' recommendations during the trial period.

Statistical analysis

Comparisons on the incidence of ECF and other tick-borne diseases in the cattle in the four groups were analyzed by the chi-square (χ^2) test. A probability value of less than 5% ($P<0.05$) was used to denote a significant difference.

RESULTS

Seroconversions

The results of seroconversion following ECF immunization are presented in Table 1. During immunization, clinical ECF reactions occured in the vaccinated cattle on only farm 1.

Tick-borne diseases during natural tick challenge

East Coast fever

The number of ECF cases in the different treatment groups are presented in Table 2. The number of ECF cases is significantly higher ($\chi^2 = 16.9$, $P > 0.05$) in the non-immunized animals (24 cases) than in the immunized animals (four cases). However, when analyzed at a farm level, the difference is only significant in the animals on farm 1 ($\chi^2 = 13.3$, $P < 0.05$). Although more ECF cases were encountered among non-immunized than among immunized animals on the other two farms, the difference is not significant at 5 % probability level. There is no significant difference between the number of ECF cases in the immunized and non-immunized animals which were under strategic tick control at the site or farm level. In the tactically dipped cattle, the number of ECF cases is significantly higher ($\chi^2 = 20.8$, $P < 0.05$) among the non-immunized (19 cases) as compared to the immunized cattle (one case). However, at farm level, the difference is only significant in the animals on farms 1 and 2 ($P < 0.05$).

TABLE 1 Serological reactions of cattle following ECF immunization

Farm	Seropositive (%)		ECF reactors (%)
	Pre-immunization	Post-immunization	
Farm 1	125.9	83	(6/50) 12
Farm 2	37.0	90	0
Farm 3	37.5	80	0

TABLE 2 Numbers of tick-borne diseases in immunized and non-immunized cattle

Treatment	Tick-borne diseases		
(n = 45)	ECF	Babesiosis	Anaplasmosis
Immunized x SD	3	4	3
Immunized x TD1	3	2	
Non-immunized x SD	5	5	1
Non-immunized xTD19	3		3
Total cases	28	15	9

n – Animals per treatment group
SD – Strategic dipping
TD – Tactical dipping

Babesiosis

Babesiosis was diagnosed in all the treatment groups (Table 2). The number of cases is not significantly different between immunized (7) and non-immunized cattle (8). Similarly, there is no difference between the strategically (9) and tactically dipped cattle (6) although more cases occurred in the immunized cattle.

Anaplasmosis

A total of nine cases of anaplasmosis were diagnosed on the three farms (Table 2). There is no significant difference in the number of cases between the immunized and non-immunized animals maintained in the two tick control strategies at site or farm level.

Tick counts and tick control

Fig. 1 and 2 show the mean tick counts in the tactically and the strategically dipped cattle on the three farms. Tick burdens were generally low on the three farms between May and September although tick burdens were higher in the strategically dipped group. Generally, tick burdens started increasing in July and reached a plateau in March in the strategically dipped cattle and in January and March in the tactically dipped cattle. *Rhipicephalus appendiculatus* was the main tick species on farms 2 and 3 while *Boophilus decoloratus* was the main tick species on farm 1. Burdens by both species were however highest on farm 1 and least on farm 2. The African bont tick (*Ambylomma variegatum*) and *Rhipicephalus evertsi* occurred in very low numbers with the maximum number on a single animal being two ticks. Eight tactical acaricide applications were done on farm 1, two on farm 2 and four on farm 3.

DISCUSSION

Mutugi *et al.* (1989) demonstrated that *Theileria parva (*Marikebuni) protects against most of the *Theileria* isolates in Kenya under laboratory conditions. Field trials also showed that it was efficacious at the

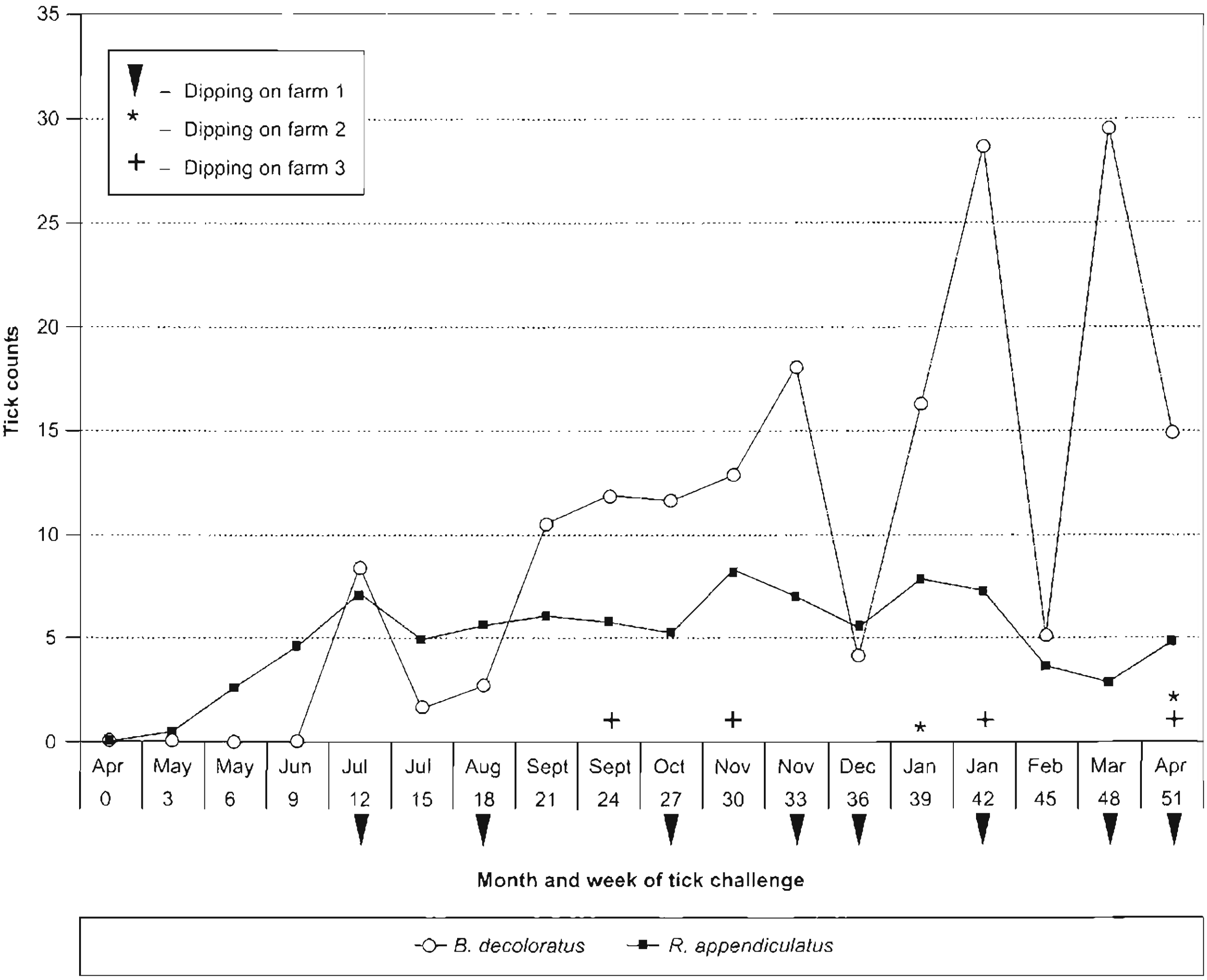

FIG. 1 Mean half body tick counts on tactically dipped cattle

Kenya coast (Mutugi *et al.* 1991) and in Central Province (Mbogo *et al.* 1994). The results emanating from the present study demonstrate its efficacy in the Trans-Nzoia District of Rift Valley Province at a location more than 800 km from where this parasite was originally isolated, and that it may be an ideal immunizing stock for use in cattle in this district. In the tactically dipped cattle, the maximum number of *Boophilus decoloratus* and *Rhipicephalus appendiculatus* on a single animal were 160 and 27 ticks respectively, while on the strategically dipped cattle, the numbers were 97 and 32, respectively. However, the maximum mean total tick counts was approximately 36 on the undipped cattle and 15 for the animals dipped every 3 weeks. For some unknown reasons, some cattle on all three farms consistently carried far more ticks than the rest of the herd. In previous immunization trials mean total tick counts of approximately 100 have been reported in undipped boran cattle (De Castro, Young, Dransfield, Cunningham & Dolan 1985) and in exotic/boran crosses (Kariuki, Muraguri & Kamau 1996). In the present study, mean tick loads were consistently higher in the tactically dipped cattle than in those under strategic dipping. Similar results have been found in other studies (De Castro *et al.* 1985; Amoo, Dipeolu, Capstick, Munyinyi, Gichuru & Odhiambo 1993). Although our study did not include monitoring for productivity, the results obtained by these other workers showed that there were no differences in live weight gains and milk output in immunized animals under intensive and relaxed dipping regimes. There was no significant difference in the number of ECF cases among immunized animals dipped every 3 weeks and those not dipped. Amoo *et al.* (1993) reported similar findings in immunized cattle maintained in intensive, relaxed or no-dipping regimes at the Kenya coast. In their study, more deaths occurred in the immunized animals that were dipped every week. They attributed this to lowering of enzootic stability due to inadequate tick challenge resulting from the intensive dipping which suggests that a moderate natural tick challenge is

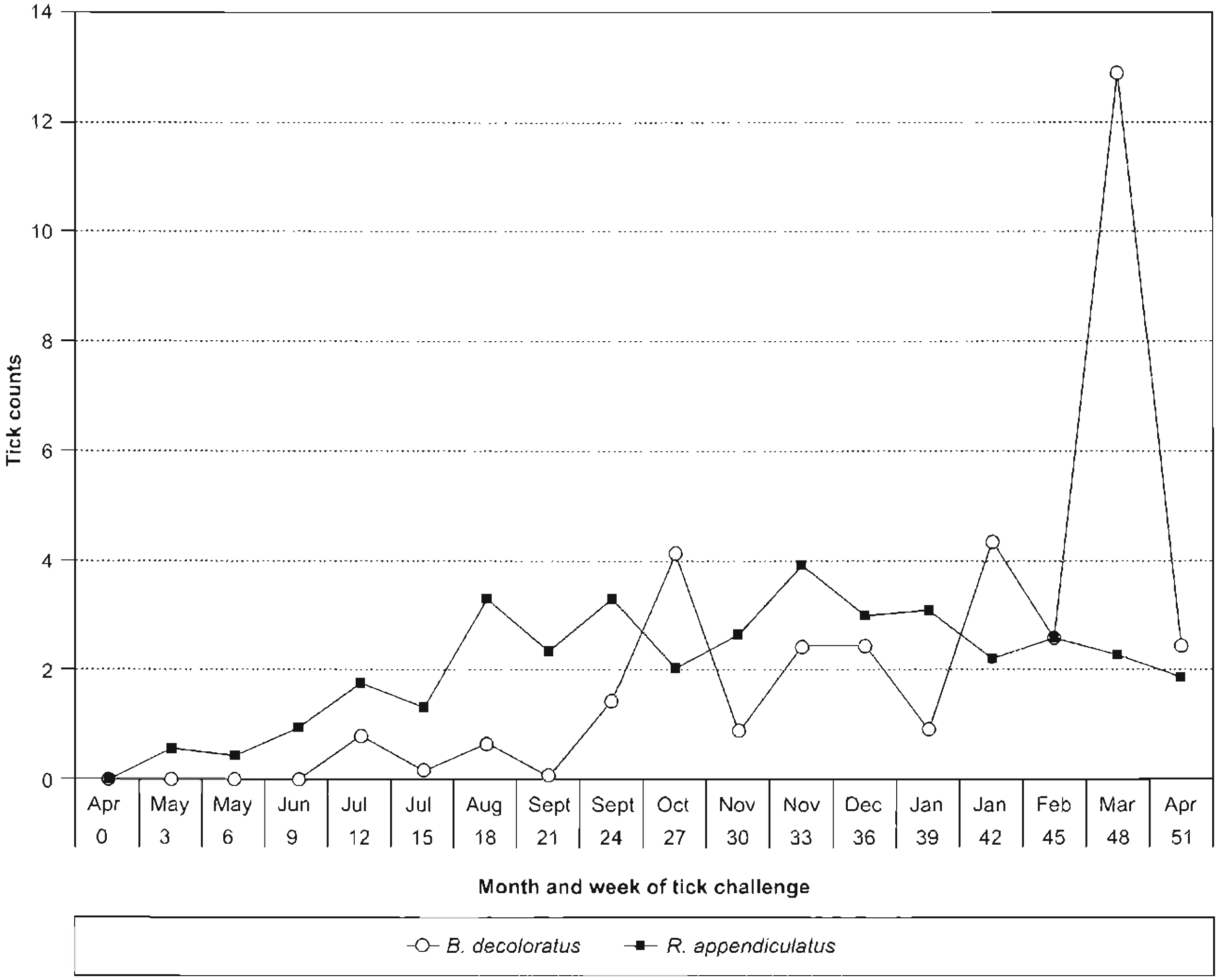

FIG. 2 Mean half body tick counts on strategically dipped cattle

necessary to maintain the immune status after immunization. Therefore, our findings indicate that a tactical dipping approach should be considered if the financial aspects of tick control are to be taken into account. Immunized beef cattle were maintained at the Kenyan coast with more limited tick control of dipping once every 3 weeks without a significant loss in cattle productivity (Morzaria *et al.* 1988). However, the degree of relaxation of acaricidal control in an area will depend on the intensity of the tick challenge and the presence of other tick-borne diseases. It is therefore essential to establish tick population dynamics for a specific area before a relaxation in tick control following ECF immunization can be advocated. Recently, computer models (DYMEX) have been developed for the prediction of seasonal population dynamics of a number of economically important ticks. The predicted seasonal pattern for *Rhipicephalus appendiculatus* in the study area by Kariuki, Kiara, Muraguri & Mwangi (1997) using this tool was very similar to our findings and such predictions could prove to be invaluable. In the absence of adequate information on tick population dynamics, however, it is considered that dipping at intervals of 3 weeks after immunization would be ideal for this area.

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REFERENCES

AMOO, A.O.J., DIPEOLU, O.O., CAPSTICK, P.B., MUNYINYI, D.M., GICHURU, L.N. & ODHIAMBO, T.R. 1993. Ixodid ticks (Acari: Ixodidae) and livestock production: Effect of varying acaricide treatments on ticks and productivity in East Coast fever immunized weaner and dairy cattle. *Journal of Medical Entomology*, 30:503–512.

BURRIDGE, M.J. & KIMBER, C.D. 1972. The indirect fluorescent antibody test for experimental East Coast fever (*Theileria parva* infection in cattle). Evaluation of a cell culture schizont antigen. *Research in Veterinary Science*, 13:451–455.

BURRIDGE, M.J., MORZARIA, S.P., CUNNINGHAM, M.P. & BROWN, C.G.D. 1972. Duration of immunity to East Coast fever (*Theileria parva*) infection of cattle. *Parasitology*, 64 :511–515.

DE CASTRO, J.J., YOUNG, A.S., DRANSFIELD, R.D., CUNNINGHAM, M.P. & DOLAN, T.T. 1985. Effects of tick infestation on Boran (*Bos indicus*) cattle immunized against theileriosis in an endemic area of Kenya. *Research in Veterinary Science*, 39:279–288.

DOLAN, T.T., YOUNG, A.S., LEITCH, B.L. & STAGG, D.A. 1984. Chemotherapy of East Coast fever: Parvaquone treatment of clinical disease induced by isolates of *Theileria parva*. *Veterinary Parasitology*, 15:103–116.

IRVIN, A.D., DOBBELAERE, D.A.E., MWAMACHI, D.M., MINAMI, T., SPOONER, P.R. & OCAMA, J.G.R. 1983. Immunization against East Coat fever: correlation between monoclonal antibody profiles of *Theileria parva* stocks and cross immunity *in vivo*. *Research in Veterinary Science*, 35:341–346.

JAETZOLD, R. & SCHIMIDT, H. 1983. *Farm management handbook of Kenya*. 11. Ministry of Agriculture, Kenya.

KARIUKI, D.P., MURAGURI, G.R. & KAMAU, M. 1996. Reduced acaricide application after immunization against East Coast fever by the infection and treatment method. *East African Agricultural and Forestry Journal*, 61:255–259

KARIUKI, D.P., KIARA, H.K., MURAGURI, R.G. & MWANGI, E.K. 1997. Designing control strategies for livestock ticks in Kenya using computer models. *International Tick Modelling Workshop*. ICIPE, Nairobi, 9–19 September 1997.

LAWRENCE, J.A., FOGGIN, C.M. & NORVAL, R.A.I. 1980. The effects of war on the control of diseases of livestock in Rhodesia (Zimbabwe). *Veterinary Record*, 107:82–85

MBOGO, S.K., KARIUKI, D.P., NGUMI, P.N., RUMBERIA, R.M., LINYONYI, A., WANJOHI, J.M. & LESAN, A.C. 1994. Immunization of small-holder cattle against East Coast fever in Kiambu District, Kenya. *East African Agricultural and Forestry Journal*, 60:41–44.

MORZARIA, S.P., IRVIN, A.D., TARACHA, E. & SPOONER, P.R. 1985. East Coast fever immunization in Coast Province of Kenya, in *Immunization against theileriosis in Africa*, edited by A.D. Irvin, Nairobi: Ilrad.

MORZARIA, S.P., IRVIN, A.D., TARACHA, E., SPOONER, P.R., VOIGHT, W.P., FUJINAGA, T. & KATENDE, J. 1987. Immunization against East Coast fever: The use of selected stocks of *Theileria parva* for immunization of cattle exposed to field challenge. *Veterinary Parasitology*, 23:23–41.

MORZARIA, S.P., IRVIN, A.D., WATHANGA, J., D'SOUZA, D., KATENDE, J., YOUNG, A.S., SCOTT, J. & GETTINBY, G. 1988. The effect of East Coast fever immunization and different acaricidal treatments on the productivity of beef cattle. *Veterinary Record*, 123: 313–320.

MUKHEBI, A.W. 1992. Economic impact of theileriosis and its control in Africa, in *The epidemiology of theileriosis in Africa*, edited by R.A.I. Norval, B.D. Perry & A.S.Young. London: Academic Press.

MUKHEBI, A.W., KARIUKI, D.P., MUSSUKUYA, E., MULLINS, G., NGUMI, P.N., THORPE, W. & PERRY. B.D. 1995. Assessing the economic impact of immunization against East Coast fever: a case study in Coast Province, Kenya. *Veterinary Record*, 137:17–22.

MUTUGI, J.J., YOUNG, A.S., MARITIM, A.C., NDUNGU, S.G., MINING, S.K., LINYONYI, A., NGUMI, P.N., LEITCH, B.L., MORZARIA, S.P. & DOLAN, T.T. 1989. Immunization of cattle against theileriosis in Coast Province, Kenya: Laboratory evaluation of of a large *Theileria parva* stabilate for use in 'infection and treatment' immunization in the field. *Research in Veterinary Science*, 47:170–177.

MUTUGI, J.J., NDUNGU, S.G., LINYONYI, A., MARITIM, A.C., MINING, S.K., NGUMI, P.N., KARIUKI, D.P., LEITCH, B.L., D'SOUZA, D., MALOO, S. & LOHR, K.F. 1991. Responses to a vaccine trial for East Coast fever in two cattle herds at the Kenyan coast. *Preventive Veterinary Medicine*, 21:215–235.

NOLAN, J. 1990. Acaricide resistance in single and multi-host ticks and strategies for its control. *Parassitologia*, 32:145–153.

NYANGITO, H.O., RICHARDSON, J.W., MUKHEBI, A.W., MUNDY, D.S., ZIMMEL, P., NAMKEN, J. & PERRY, B.D. 1994. Whole farm economic analysis of ECF immunization strategies in Kilifi District, Kenya. *Preventive Veterinary Medicine*, 21:215–235.

RADLEY, D.E., BROWN, C.G.D., BURRIDGE, M.J., CUNNINGHAM, M.P., KIRIMI, I.M., PURNELL, R.E. & YOUNG, A.S. 1975. East Coast fever: 1. Chemoprophylactic immunization of cattle against *Theileria parva* (muguga) and five theilerial strains. *Veterinary Parasitology*, 1:35–41.

RADLEY, D.E. 1981. Infection and treatment method of immunization against theileriosis, in *Advances in the control of theileriosis*, edited by A.D. Irvin, M.P. Cunningham & A.S. Young. The Hague: Martinus Nijhoff: 227–237.

YOUNG, A.S., LEITCH, B.L., DOLAN, T.T., MBOGO, S.K., NDUNGU, S.G., GROOTENHUIS, J.G. & DE CASTRO, J.J. 1990. Evaluation of infection and treatment methods in immunization of improved cattle against Theileriosis in an endemic area of Kenya. *Veterinary Record*, 35:239–257.

YOUNG, A.S., MUTUGI, J.J., KARIUKI, D.P., LAMPARD, D., MARITIM, A.C., NGUMI, P.N., LINYONYI, A., LEITCH, B.L., NDUNGU, S.G., LESAN, A.C., MINING, S.K., GROOTENHUIS, J.G., ORINDA, G.O. & WESONGA, D. 1992. Immunization of cattle against theileriosis in Nakuru District of Kenya by infection and treatment and the introduction of unconventional tick control. *Veterinary Parasitology*, 42:225–240.